(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) Publication number: **0 594 959 A1**

(12) EUROPEAN PATENT APPLICATION

(21) Application number: **93113261.7**

(22) Date of filing: **19.08.93**

(51) Int. Cl.5: **C12Q 1/68**, C07H 21/04, C12P 19/34

(30) Priority: **28.08.92 US 938084**

(43) Date of publication of application:
04.05.94 Bulletin 94/18

(84) Designated Contracting States:
BE CH DE DK ES FR GB GR IE IT LI LU NL PT

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(54) A chemiluminescent method for the quantitation of human DNA.

(57) The present invention provides methods and reagents for the estimation of the quantity of human DNA contained in a sample. Immobilized sample DNA is hybridized to a biotinylated oligonucleotide probe that hybridizes to a human genomic or mitochondria DNA sequence. The subsequent binding of streptavidin-horseradish peroxidase to the bound probe allows for chemiluminescent detection using a luminol-based reagent and X-ray film.

In addition, the present invention provides methods and reagents to assess the quality of DNA contained in a sample. The sample is first size fractionated by agarose gel electrophoresis, and then immobilized, hybridized to a biotinylated oligonucleotide probe, and detected using the chemiluminescent method as used in the quantity estimation methods of the present invention.

EP 0 594 959 A1

In addition, the present invention provides methods of evaluating the quality of DNA contained in a sample. The DNA is size fractionated by gel electrophoresis, immobilized on a membrane, hybridized with a biotinylated probe that hybridizes to a repeated human genomic sequence, and detected using a chemiluminescent assay. In one embodiment, the repeated human sequence is the human alpha satellite locus, D17Z1. The quality of the DNA is estimated from the fragment size pattern observed.

The present invention also provides biotinylated oligonucleotide probes that hybridize to sequences within the human alpha satellite locus, D17Z1, and to sequences within the mitochondrial control region for use in the methods provided.

The present invention also provides kits containing reagents used in the methods of the present invention.

Figure 1 illustrates the results of a DNA quantity assay as described in Example 2. Sample DNA was immobilized on a nylon membrane and hybridized with the probe, SW49 (SEQ ID No. 1). Bands were visualized using chemiluminescent detection with a 15 minute exposure to film Column "S" is a human genomic DNA titration series ranging from 10 to 0.15 nanograms of DNA. Columns 1-3 are samples in which the DNA quantity was unknown. The sources of the extracted DNA samples in columns 1-3 were as follows: 1A-1E were bloodstains, 1F-2C were whole blood, 2D-3B were single hairs, 3C-3E were buccal samples, 3F was 1 µg of cow DNA, 3G was 1 µg mouse DNA; no sample was added to 3H.

Figure 2 illustrates the results of a DNA quality assay as described in Example 3. Figure 2A illustrates a photograph of a 1% agarose gel. Human genomic DNA (14 nanograms) was boiled in either 5% Chelex or water for 0, 1, 3, or 8 minutes and then subjected to electrophoresis. DNA size markers were run concurrently on the gel and are indicated by "M". The DNA was subsequently transferred to a nylon membrane, hybridized with the probe, SW49 (SEQ ID No. 1), and visualized by chemiluminescent detection with a 15 minute exposure to film Figure 2B illustrates the resulting photograph. Photograph labels are as in Figure 2A.

The term "sample" as used herein refers to any substance containing or presumed to contain nucleic acid including, but not limited to, tissue or fluid isolated from one or more individuals, in vitro cell culture constituents, as well as evidential, clinical, archival, and ancient samples.

The terms "oligonucleotide" and "nucleic acid" as used herein refer to molecules comprising two or more deoxyribonucleotides or ribonucleotides. The exact size will depend upon many factors, which in turn depend on the ultimate function or use of the oligonucleotide. The terms refer to both single- and double-stranded DNA and RNA. Oligonucleotides may be derived by any suitable technique including, but not limited to, isolation of an existing or natural sequence, chemical synthesis, DNA replication or amplification, reverse transcription, or a combination thereof. Chemical synthesis methods may include, for example, the phosphotriester method described by Narang et al., 1979, Methods in Enzymology 68:90,the phophodiester method described by Brown et al., 1979, Methods in Enzymology 68:109, the diethylphosphoramidite method described by Beaucage et al., 1981, Tetrahedron Letters 22:1859, and the solid support method disclosed in U.S. Patent No. 4,458,066. Oligonucleotide synthesis is described in Levenson and Chang, 1990, in PCR Protocols, Innis et al. (eds.), Academic Press, New York:99-112.

The term "subsequence" as used herein refers to a nucleotide sequence which is wholly contained within another nucleotide sequence. As defined, a sequence is also a subsequence of itself. As used herein, a subsequence suitable as a hybridization probe is about 10-140 nucleotides in length and preferably 40-130 nucleotides in length.

The term "quality" as used herein refers to the degree of degradation of a DNA sample. A high quality sample contains DNA which has undergone little or no degradation. The quality of a DNA sample can be assessed by measuring the molecular weight of the sample DNA by gel electrophoretic size fractionation. For example, using a 1% agarose gel, a high quality sample of human genomic DNA migrates with a 20 kb DNA marker and forms a relatively tight band when visualized either by ethidium bromide staining or by the methods of the present invention, whereas in a low quality human DNA sample, degradation of the DNA yields fragments of varying length which appear as a smear of lower molecular weight DNA, i.e., less than 20kb.

The terms "probe" and "oligonucleotide probe" as used herein refer to labeled oligonucleotides which are sufficiently complementary to a specific target sequence contained in a DNA sample to form a stable hybridization duplex with the target sequence. The hybridization is under stringent conditions. Stringent hybridization conditions are well known in the art and are described, for example, in Sambrook et al., 1989, Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor Laboratory, New York. The term "hybridizing region" refers to that region of an oligonucleotide probe which is complementary to, and therefore hybridizes to, the target sequence. Although the hybridizing region typically refers to the entire otigonucleotide, the probe may include additional nucleotide sequences which function, for example, as the

In a preferred DNA quantitation method, a DNA sample is immobilized on a nylon membrane before hybridizing with the labeled probes as described in Example 2. A commercially available apparatus (e.g., the Convertible, GIBCO BRL, Gaithersburg, MD, USA) may be used to immobilize the DNA sample on the membrane in a specified location. Thus, a large number of samples can be immobilized on the same membrane in a defined array. Simultaneous hybridization of numerous samples can be effected by immersion of the membrane in a hybridization buffer containing the quantitation probe. The methods of the present invention are particularly suited to applications in which a large number of samples need to be analyzed on a routine basis, such as in a commercial environment.

In a preferred DNA quality assay method, after size fractionation of the DNA sample by agarose gel electrophoresis, the fractionated DNA is transferred to a nylon membrane before hybridizing with the probes of the present invention. The DNA may be transferred using a commercially available apparatus (e.g., the Posiblot transfer system, Stratagene, La Jolla, CA, USA). After the DNA is transferred to the nylon membrane, the DNA may be fixed to the membrane by baiting at 80 • C, as described in Example 3, or by crosslinking of the thymidine residues to the membrane by UV irradiation (Church and Gilbert, 1984, Proc. Natl. Acad. Sci. USA 81:1991-1995).

Reagents employed in the methods of the present invention can be packaged into kits. Kits include the labeled oligonucleotide probe or, if unlabeled, specific labeling reagents may be included. The kits may also include suitably packaged reagents and materials needed for DNA immobilization and detection, such membranes, buffers, enzymes, DNA standards, and a hybridization tray, as well as instructions for conducting the assay.

The following examples are offered by way of illustration and are not intended to limit the invention in any manner.

Example 1

Probes

The probes of the present invention are complementary to a region contained within the 2.7 kb D17Z1 locus. Each oligonucleotide is bound to a biotin molecule at the 5' end either directly or through a phosphorarmidite "spacer" molecule. Probes SW49 (SEQ ID No. 1), SW1000 (SEQ ID No. 1), SW1001 (SEQ ID No. 1), SW1002 (SEQ ID No. 1), SW1003 (SEQ ID No. 1), SW1004 (SEQ ID No. 1), and SW1005 (SEQ ID No. 1) were constructed with the identical oligonucleotide sequence, differing in the details of the label. The nucleotide sequence common to each of the probes is provided below and in the Sequence Listing.

SEQ ID No.	Sequence
1	5'TAGAAGCATTCTCAGAAACTACTTTGTGATGATTGCATTC

Oligonucleotide synthesis was performed on an automated DNA synthesizer (either Millige/Biosearch 8750, Applied Biosystems 394; or Eppendorf Biotronik D) on a micromole scale using 500 angstrom controlled-pore glass supports and O-cyanoethyl N,N-diisopropyl phosphoramidites as described in Beaucage and Caruthers, 1981, Tetrahedron Letters, 22:1859-1862 and Sinha et al., 1984, Nucleic Acids Research, 12:4539-4557. Supports and phosphoramidite derivatives of dA, dC, dG, and T were obtained commercially from Millipore/Waters, Bedford, MA, USA; or Cruachem, Sterling, VA, USA. Biotin and "Spacer" phosphoramidites were obtained from Glen Research, Sterling, VA. Two biotin reagents were used: Biotin Phosphoramidite and BioTEG Phosphoramidite. These two biotin reagents differ in that the BioTEG product includes a longer spacer separating the biotin from the oligonucleotide. Oligonucleotides were synthesized with the terminal dimethoxytrityl group left intact and were purified by lipophilic selection using solid-phase extraction cartridges (PREP-NENSORB, Dupont). Biotinylation and oligonucleotide purification are described in Misiura et al., 1990, Nucleic Acids Research 18:4345-4354; Alves et al., 1989, Tetrahedron Letters, 30:3089-3092; and Pon, 1991, Tetrahedron Letters 32:1715-1718.

Quantitation probes SW49 (SEQ ID No. 1), SW1000 (SEQ ID No. 1), SW1001 (SEQ ID No. 1), SW1002 (SEQ ID No. 1), SW1003 (SEQ ID No. 1), SW1004 (SEQ ID No. 1), and SW1005 (SEQ ID No. 1) are shown schematically in Table 1, below. In Table 1, B1 refers to Biotin Phosphoramidite, B2 refers to BioTEG Phosphoramidite, Spacer refers to a phosphoramidite spacer. Oligo refers to the oligonucleotide (SEQ ID No. 1) common to the probes. mtOligo refers to mitochondrial DNA oligonucleotides (sequences provided

SW57	10	5'TAGAAGCATTCTCAGAAACTACTTTGTGATGATTGCATTCA AGTCACAGAGTTGAACATTCCCTTTGACAGAGCAGTTTG
SW58	11	5'TAGAAGCATTCTCAGAAACTACTTTGTGATGATTGCATTCAAGTCAC AGAGTTGAACATTCCCTTTGACAGAGCAGTTTGGAAACTCTCTTTGTGTA GAA

The oligonucleotide probe sequences are subsequences of the D17Z1 constituent monomers. SW33 (SEQ ID No. 4) is a subsequence of monomer 11, SW34 (SEQ ID No. 5) is a subsequence of monomer 12, and SW35 (SEQ ID No. 6) is a subsequence of monomer 13. The other oligonucleotide sequences are subsequences of one of the above 3 oligonucleotides. SEQ ID No. 1, SW56 (SEQ ID No. 7), and SW59 (SEQ ID No. 8) are non-overlapping subsequences of SW33 (SEQ ID No. 4). SEQ ID No. 1 is a subsequence of SW52 (SEQ ID No. 9), which is a subsequence of SW57 (SEQ ID No. 10), which is a subsequence of SW58 (SEQ ID No. 11), which are all subsequences of SW33 (SEQ ID No. 4). SW31 (SEQ ID No. 2) is a subsequence of SW34 (SEQ ID No. 5), and SW32 (SEQ ID No. 3) is a subsequence of SW35 (SEQ ID No. 6).

Additional probes of the present invention are complementary to various conserved sequences within the mitochondrial control region.

Probe	SEQ ID No.	Sequence
RR64	12	5'GGCGGTATGCACTTTTAACAGTCACCCCCCAACTAACAC
RR65	13	5'GTCTTTAACTCCACCATTAGCACCCAAAGCTAAGATTCTA
RR66	14	5'CGTGAAATCAATATCCCGCACAAGAGTGCTACTCTCCTCG
RR67	15	5'GAACTGTATCCGACATCTGGTTCCTACTTCAGGGTCATAAAGC
RR68	16	5'GACATCACGATGGATCACAGGTCTATCACCCTATTAACCAC
RR69	17	5'CATCCTCCGTGAAATCAATATCCCGCACAAGAGTGCTAC
RR70	18	5'GTCTTTAACTCCACCATTAGCACCCAAAGC
RR71	19	5'CTCCACCATTAGCACCCAAAGCTAAGATTC
RR72	20	5'GTATCCGACATCTGGTTCCTACTTCAGGGTC
RR70 (SEQ ID No. 18) and RR721 (SEQ ID No. 19) are overlapping subsequences of RR65 (SEQ ID No. 13). RR72 (SEQ ID No. 20) is a subsequence of RR67 (SEQ ID No. 15). RR66 (SEQ ID No. 14) and RR69 (SEQ ID No. 17) overlap.		

Example 2

DNA Quantity Estimation

To estimate the quantity of DNA in a sample, extracted sample DNA was immobilized on a nylon membrane along with a titration series of a human genomic DNA standard and hybridized to a biotinylated probe, SW49 (SEQ ID No. 1). The synthesis of the probe was as described in Example 1, above. Hybridization was visualized using a chemliuminescence detection protocol. The quantity of DNA present in the sample was estimated by comparison of the hybridization signal obtained from the sample DNA to those obtained from the DNA standards. Details of the experimental protocol are as follows.

The quantities of human DNA in extracts from 5 human bloodstain samples, 6 human whole blood samples, 7 human hair samples, and 3 human buccal samples were estimated. In addition, samples consisting of 1 µg cow DNA and 1 µg mouse DNA were also used as a test of probe specificity. DNA was extracted from samples either by the Chelex method described in Walsh et al., 1991, supra., or by a salting out method as described in Miller et al., 1988, Nucl. Acids Res. 6(3): 1215. In the Chelex method, a 3 mm^2 bloodstain, a buccal scraping, or a 1 cm hair root section were incubated in 200 µl 5% Chelex at 56°C, followed by boiling for 8 minutes.

Sample	Source	Quantity (ng)
1A	Bloodstain	2.24
1B	"	0.26
1C	"	1.07
1D	"	0.88
1E	"	1.74
1F	Whole Blood	0.83
1G	"	1.09
1H	"	7.17
2A	"	6.32
2B	"	1.32
2C	"	2.33
2D	Hair	0.28
2E	"	0.94
2F	"	0.52
2G	"	0.68
2H	"	2.49
3A	"	6.34
3B	"	1.64
3C	Buccal	1.46
3D	"	1.49
3E	"	1.00
3F	Cow DNA	0
3G	Mouse DNA	0

To estimate the quantity of mitochondrial DNA in a sample, the protocol described above for quantitation of nuclear DNA is used with the following exceptions:

1. Prehybridization is performed at 46 • C instead of 50 • C.
2. Hybridization is performed at 46 • C with 20 pmoles of the probe RR70 (SEQ ID No. 18).

A commercially available preparation of placental DNA (SIGMA) is diluted and used for the DNA standards. Since the extracted placental DNA contains a mixture of nuclear and mitochondrial DNA, the standards can be used for both types of quantity estimates. Mitochondrial DNA is the minor component of the total DNA preparation and is present in an unknown quantity. Therefore, to use placental DNA (or other total DNA) preparations as a standard for the mitochondrial DNA quantitation assay, the amount of mitochondrial DNA present must be determined. This value can be obtained using a purified mitochondrial DNA preparation that has been quantitated spectrophotometrically. Dilutions of the purified DNA can then be hybridized with the mitochondrial DNA-specific probe as described above at the same time as the total DNA dilutions, and the signal intensities can be compared to determine the quantity of mitochondrial DNA in the total DNA preparation. Obviously, a purified mitochondrial DNA sample would be the ideal quantitation standard, but the procedure for isolating mitochondrial DNA is extremely time consuming, expensive, and provides a very low-yield.

Example 3

DNA Quality Estimation

Purified human genomic DNA was diluted to 2 ng/µl in both 5% Chelex and glass distilled water, and then boiled for 0, 1, 3, or 8 minutes in a boiling water bath. Seven µl (14 ng) of each sample was subjected to electrophoresis on a 1% agarose gel containing 0.5 µg/ml ethidium bromide in 1x TBE for 30 minutes at 100 volts. The gel was photographed, soaked in 0.25 M HCl for 15 minutes to depurinate the DNA, and then soaked in 0.5 N NaOH, 1.5 M NaCl for 10 minutes to denature the DNA. The DNA was transferred to a Biodyne B membrane using the Posiblot transfer system (Stratagene, La Jolla, CA, USA). Transfer was performed at 75 mm Hg for 1 hour using 10x SSPE as the transfer buffer. The membrane was baked in a vacuum oven for 15 minutes at 80 • C to fix the DNA. The membrane was wetted with 2x SSPE and then soaked in 15% hydrogen peroxide for 2 minutes. Hybridization and detection of bound SW49 probe (SEQ ID No.1) was performed essentially as described in Example 1, above, except that the blot was exposed to film for 30 minutes.

Example 6

Preferred Method for Determining the Quantity of DNA in a Sample (Protocol)

Slot Blot

1. Add 1 to 5 µl of each DNA sample to 150 µl of spotting buffer (0.4 N NaOH, 25 mM EDTA, 0.0015 % Bromophenol Blue). Also add the following quantities of DNA standard (in 5 µl) to 150 µl of spotting buffer: 10, 5, 2.5, 1.2, 0.6, 0.3, 0.15 ng.
2. Pre-wet Biodyne B membrane in 50 mL of 0.4 N NaOH, 25 mM EDTA (5-30 minutes).
3. Place the membrane in the slot blotter, and pipette the entire volume for each sample into the wells. Apply spotting buffer containing no DNA to some of the empty wells as a negative control. Turn on the vacuum only after all samples have been applied.
4. Begin the pre-hybridization step immediately (see below).

Hybridization and Detection

1. Pre-Hybridization: Place the membrane in 150 mL of pre-warmed 5X SSPE, 0.5% SDS. Then add 5 mL of 30% H_2O_2. Shake in a water bath (70 rpm) for 15 minutes at 50 °C.
2. Hybridization: Incubate in 30 mL of 5X SSPE, 0.5% SDS containing 20 pmoles SW1004, for 20 minutes at 50 °C in a shaking water bath (70 rpm).
Rinse: Briefly rinse in 1.5X SSPE, 0.5% SDS.
3. Stringent Wash/Conjugation: Incubate in 30 mL of 1.5X SSPE, 0.5% SDS containing 90 µl SA-HRP, for 10 minutes at 50 °C in a shaking water bath (70 rpm).
Rinse: Briefly rinse in 1.5X SSPE, 0.5% SDS.
4. Wash: Incubate in 150 mL of 1.5X SSPE, 0.5% SDS at room temperature for 15 minutes on an orbital shaker (100-125 rpm).
Rinse: Briefly rinse in approximately 150 mL of 0.1 M NaCitrate, pH 5.
5. ECL: Add 10 mL ECL reagent A to 10 mL ECL reagent B. Shake the membrane in the ECL reagents for exactly 1 minute at room temperature.
6. Expose Film: Place the membrane on the plastic side of benchkote and place Saran Wrap over the membrane. Use a paper towel to smooth out any wrinkles in the Saran Wrap. Expose to Hyperfilm or Kodak XAR5 film for 15 minutes.

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 40 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

TAGAAGCATT CTCAGAAACT ACTTTGTGAT GATTGCATTC 40

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 40 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

CACTATTTGT AGAATGTGCA AGTGGATATT TAGGCCTCTC 40

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 131 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CAGTAGCATT CACAGAAAAC TCTTGGTGAC GACTGAGTTT AACTCACAGA GCTGAACATT 60
CCTTTGGATG GAGCAGTTTC GAAACACACT ATTTGTAGAA TGTGCAAGTG GATATTTAGG 120
CCTCTCTGAG G 131

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 130 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

CAGAAGCATT CTCAGAACCT TCTTCGTGAT GTTTGCATTC AACTCACAGT GTTGAACCTT 60
TCTTTGATAG TTCAGGTTTG AAACGGTCTT TCTGTAGAAA CTGCAAGTAG ATATTTGGAC 120
CGCTCTGAGG 130

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 60 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

TAGAAGCATT CTCAGAAACT ACTTTGTGAT GATTGCATTC AAGTCACAGA GTTGAACATT 60

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 80 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

TAGAAGCATT CTCAGAAACT ACTTTGTGAT GATTGCATTC AAGTCACAGA GTTGAACATT 60
CCCTTTGACA GAGCAGTTTG 80

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 40 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

GTCTTTAACT CCACCATTAG CACCCAAAGC TAAGATTCTA 40

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 40 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

CGTGAAATCA ATATCCCGCA CAAGAGTGCT ACTCTCCTCG 40

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 39 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

CATCCTCCGT GAAATCAATA TCCCGCACAA GAGTGCTAC 39

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

GTCTTTAACT CCACCATTAG CACCCAAAGC 30

4. An oligonucleotide probe of any one of claims 1-3, wherein said nucleic acid sequence is selected from the group consisting of SEQ ID No. 1, and a sequence complementary to SEQ ID No. 1.

5. An oligonucleotide probe of claim 4 selected from the group consisting of probes SW49, SW1000, SW1001, SW 1002, SW1003, SW1004 and SW1005, which probes each have the nucleotide sequence of SEQ ID No. 1 but which probes each consist of a different label moiety.

6. A method of quantitating the amount of DNA in a sample containing DNA comprising:
(a) immobilizing said DNA and a known quantity of control DNA in discrete locations on a membrane;
(b) contacting said membrane with a solution containing a probe that hybridizes to a repeated human genomic sequence, which sequence is the D17Z1 locus, under condition such that hybridization duplexes form only if said DNA contains a sequence completely complementary to said probe, wherein said probe comprises a nucleic acid sequence, said sequence consisting of between about 10-140 nucleotides in length selected from a member of the group consisting of SEQ ID Nos. 1-11 and sequences complementary thereto;
(c) detecting the presence of hybridization duplexes using an assay which generates a signal whose intensity depends on the number of hybridization duplexes detected; and
(d) estimating the amount of DNA in said sample relative to the amount of said control DNA from said signal intensities.

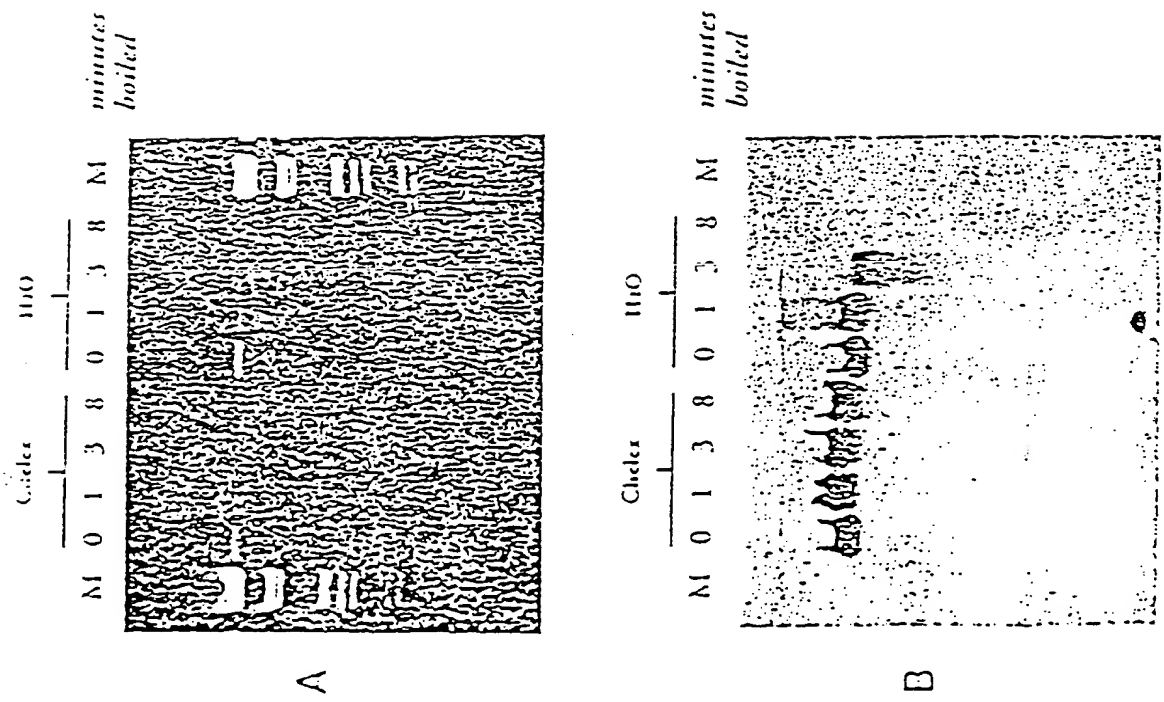
7. The method of claim 6, wherein said nucleic acid sequence is selected from the group consisting of SEQ ID Nos. 1-11 and sequences complementary thereto.

8. A method of quantitating the amount of DNA in a sample containing DNA comprising:
(a) immobilizing said DNA and a known quantity of control DNA in discrete locations on a membrane;
(b) contacting said membrane with a solution containing a probe that hybridizes to a sequence contained within the control region of the mitochondrial genome under condition such that hybridization duplexes form only if said DNA contains a sequence completely complementary to said probe, wherein said probe comprises a nucleic acid sequence, said sequence consisting of between about 10 and 140 nucleotides selected from a member of the group consisting of SEQ ID Nos. 12-20 and sequences complementary thereto;
(c) detecting the presence of hybridization duplexes using an assay which generates a signal whose intensity depends on the number of hybridization duplexes detected; and
(d) estimating the amount of DNA in said sample relative to the amount of said control DNA from said signal intensities.

9. The method of claim 8, wherein said nucleic acid sequence is selected from the group consisting of SEQ ID Nos. 12-20 and sequences complementary thereto.

10. A kit fro quantitating the amount of human DNA in a sample, said kit comprising an oligonucleotide probe of any one of claims 1-4.

Figure 2


Chelex
H2O
M 0 1 3 8 0 1 3 8 M
minutes boiled
A
Chelex
H2O
M 0 1 3 8 0 1 3 8 M
minutes boiled
B